AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (currently amended) A method for generating a molecular profile of genomic DNA by hybridization of a genomic DNA target to ~~an~~ a plurality of immobilized nucleic acid probes~~probe~~, comprising ~~the following steps~~:

(a) providing ~~a~~ the plurality of nucleic acid probes comprising a plurality of immobilized nucleic acid segments in an array with each probe at a known location;

(b) ~~providing~~ contacting the immobilized probes with a sample of target nucleic acid comprising fragments of genomic nucleic acid labeled with a detectable moiety, wherein each labeled fragment consists of a length smaller than about 200 bases, ~~;~~ and ~~(c)~~ the contacting is ~~the genomic nucleic acid of step (b) with the immobilized probes of step (a)~~ under conditions allowing hybridization of the target nucleic acid to the probe nucleic acid; and

(c) observing an amount and location of labeled genomic nucleic acid hybridized to each immobilized probe, to detect regions of amplification, deletion and unique sequences in the sample, thereby generating a molecular profile of the sample genomic nucleic acid.

2. (original) The method of claim 1, wherein each labeled fragment consists of a length no more than about 150 bases.

3. (original) The method of claim 2, wherein each labeled fragment consists of a length no more than about 100 bases.

4. (original) The method of claim 3, wherein each labeled fragment consists of a length no more than about 50 bases.

5. (original) The method of claim 4, wherein each labeled fragment consists of a length no more than about 30 bases.

6. (currently amended) The method of claim 2 ~~3~~, wherein each labeled fragment consists of a length between about 30 bases and about 150 bases.

7. (previously amended) The method of claim 1, wherein the sample of target genomic nucleic acid is prepared using a procedure selected from the group consisting of random priming, nick translation, and amplification, of a sample of genomic nucleic acid to generate segments of target genomic nucleic acid; followed by a step comprising fragmentation or enzymatic digestion, or both, of the segments to generate a sample of target genomic nucleic acid consisting of sizes smaller than about 200 bases.

8. (previously amended) The method of claim 7, wherein the random priming, nick translation, or amplification, of the sample of genomic nucleic acid to generate segments of target genomic nucleic acid incorporates detectably labeled base pairs into the segments.

9. (previously amended) The method of claim 8, wherein the detectable label comprises Cy3™ or Cy5™.

10. (currently amended) The method of claim 1, ~~wherein~~ further comprising prior to step (b), fragmenting the sample of target genomic nucleic acid ~~is prepared using a procedure comprising fragmentation of a genomic DNA~~ to sizes smaller than about 200 bases by DNase enzyme digestion ~~of the segments~~.

11. (currently amended) The method of claim 1, ~~wherein~~ further comprising prior to step (b), fragmenting the sample of target genomic nucleic acid ~~is prepared using a procedure comprising fragmentation of a genomic DNA~~ to sizes smaller than about 200 bases by applying shearing forces sufficient to fragment genomic DNA followed by DNase enzyme digestion of the sheared DNA.

12. (original) The method of claim 1, wherein the conditions allowing hybridization of the target nucleic acid to the probe nucleic acid comprise stringent hybridization conditions.

13. (original) The method of claim 12, wherein the stringent hybridization conditions comprise a temperature of about 60°C to about 65°C.

14. (original) The method of claim 1, wherein the target nucleic acid consists essentially of DNA derived from a human.

15. (currently amended) The method of claim 1, further comprising prior to step (a), choosing a plurality of DNA probes representing a defined part or substantially an entire chromosome to be immobilized, wherein observing the labeled fragments is observing hybridization of the sample of target genomic nucleic acid ~~comprises~~ to sequences representing ~~a~~ the defined part of or substantially ~~an~~ the entire chromosome.

16. (currently amended) The method of claim 15, wherein choosing the plurality of DNA probes is choosing a defined part or substantially an entire genome, and observing the labeled fragments is observing hybridization of the sample of target genomic nucleic acid ~~comprises~~ to sequences representing substantially ~~an~~ the entire genome.

17. (previously amended) The method of claim 15 or 16, wherein the chromosome or genome is derived from a human.

18-66 (withdrawn).

67. (currently amended) The method of claim 1, wherein the sample of target genomic nucleic acid comprises ~~representing~~ at least one chromosome.

68. (currently amended) The method of claim 1, wherein the sample of target genomic nucleic acid comprises ~~representing~~ a complete genome.

REMARKS

Claims 1, 6, 10-11, 15-16, and 67-68 are here amended. Support for amendment of claim 1 can be found in the claim as originally filed, and at page 14, lines 23-25 and p. 17, line 9. Claims 6 and 67-68 are amended to correct clerical errors. Support for the amendment to claims 15-16 is found in claim 1, and in these claims as originally filed.

No new matter is added by the amendments herein to the claims. After entry of these amendments, claims 1-17 and 67-68 remain pending in the application.

The Office Action dated April 24, 2003 has withdrawn the following rejections: rejection of claims 7-11 and 17 under 35 U.S.C. §112, second paragraph; rejection of claims 1-12, 15, and 16 under 35 U.S.C. §102 in view of Lockhart et al.; rejection of claim 13 under 35 U.S.C. §103 over Lockhart in view of Anderson et al.; and rejection of claims 14 and 17 under 35 U.S.C. §103 in view of Lockhart.

Claim objections

Claims 1 and 6 are here amended to address the Examiners remarks in paragraphs 5 and 6 on p. 4 of the Office Action. Claim 1 as amended now recites step (c) which is an observing step, and the results obtained by a user from this step cause the claim now to be consistent with the preamble. Claim 6 has been amended to depend from claim 3, which corrects a clerical error in the claim as filed and provides a correct relationship of the claims.

Applicants thank the Examiner for her careful reading of the claims and for her comments resulting in these amendments, and respectfully assert that the amendments to claims 1 and 6 have made moot the objections to these claims.

Issues under 35 U.S.C. §112, second paragraph

The Office Action on page 2 maintains rejection of claims 15-16 under 35 U.S.C. §112, second paragraph.

Claims 15-16 are rejected as indefinite because of the limitation "...sequences representing substantially an entire chromosome." in claim 15, or "substantially an entire

genome" in claim 16. The Examiner discusses the description on p. 3, lines 25-31 of the specification, stating that one cannot conclude what percentage of a chromosome or genome would be considered as "substantially entire".

Applicants previously submitted that one of ordinary skill in the art of genomic assays would have known, at the time that the application was filed, that the term "substantially" with respect to an entire chromosome or genome means that the plurality of immobilized probes contain nucleic acids of a chromosome (or genome) which is almost complete or may be complete, but is not necessarily total, i.e., that "substantially" means almost but possibly not quite all.

Applicants further now assert that the extent of coverage of a chromosome (or a genome) to be obtained by a user of the present claims must necessarily be under the control of that user. To get 100% coverage of a chromosome (or genome), the user would provide a plurality of nucleic acid probes which is a larger number, than would another user who desired only to get coverage of substantially an entire chromosome (or genome). For many users, substantially an entire chromosome is sufficient, i.e., for diagnosis of many genetic disorders or cancers that result from deletion of all or of a large portion of a chromosome such as Turner syndrome (XO), probes that collectively have nucleic acid from the entire X chromosome would not be necessary to distinguish a Turner's sample from a normal (XX) sample. In this manner, a "defined part" is that portion of the chromosome (or genome) that is chosen by the user.

Because of variable needs of users such as customers of a licensee, and variable designs of the nature of the collective set of immobilized nucleic acids that constitute the probe, strict limitations such as 60% of a chromosome or 90% of a genome are not relevant as structural limitations in this art. The application as filed teaches use of a large number of different libraries of genomic clones (see p. 18, lines 13-29 that directs the user of an extensive list of references to many types of libraries, many of which are commercially available or are free). Depending on what the user wants, the members of each library are selected by the user, and so each library can collectively provide a defined part, or substantially all, or all, of a chromosome (or a genome) by choice of a discrete number of clones. The user chooses the portion, or substantially all, or all, and selects the appropriate clones. For example, a user wanting a plurality of probes having

nucleic acid of one chromosome, or substantially all or all of that chromosome, of a mammal may need to be provided with 40 clones (for a small chromosome such as human X or 21) or with about 100 or more for a large chromosome (such as human 4).

However this information is specific to the species on which the library is based, the capacity of the cloning vector used for each library, and the purpose to which the user will apply the claimed method. Because of these variations in needs of the end user, then whether the user prefers probes for the entire genome or the entire chromosome with or without overlaps, or substantially all of the genome or chromosome with small gaps, or a portion that is optimal for that user are not relevant to use of the claimed method. Because of these considerations, Applicants assert that the the claim as filed would have been clear in its meaning to one of ordinary skill in the art of genetic analysis.

Nevertheless, claims 15 and 16 are here amended to describe operation of of the method.

The Examiner on p. 4 paragraph 8 of the Office Action rejects claim 1 and its dependent claims, for failing to recite a final process step which clearly relates back to the preamble. Claim 1 as amended now includes a final process step. Applicants believe that this amendment places claim 1 in condition for allowance. Amendments here to other claims all of which depend directly or indirectly from claim further place these claims in condition for allowance.

Claims 67 and 68 are here amended to removing the word "representing", as a sample of nucleic acid as prepared as directed by the specificaton actually comprises a complete chromosome (claim 67) or a complete genome (claim 68), rather than representing these structures.

In light of the remarks and amendments herein, Applicants respectfully request withdrawal of rejection of the claims under 35 U.S.C. §112, second paragraph.

Issues under 35 U.S.C. §102

Applicants believe that it would be helpful to the Examiner to provide a description of the invention of claim 1 as here amended, prior to discussing the prior art.

Claim 1 as amended is directed to a method for generating a molecular profile of a genomic DNA by hybridization of a genomic DNA target to a plurality of immobilized ucleic acids segments in an array, such that each probe has a known location, and contacting these probes with a sample of target nucleic acid comprising fragments of genomic nucleic acid labeled with a detectable moiety. Each labeled fragment consists of a length smaller than about 200 bases. Contacting happens under conditions allowing hybridization of the target nucleic acid to the probe nucleic acid. By observing the location and amount of labeled genomic acid hybridized to the immobilized probes, regions of amplification, deletion and unique sequences are detected in the sample, which generates the molecular profile of the sample genomic nucleic acid.

Huang et al. (Human Mol. Genetics 8: 459-470, 1999)

The Examiner on p. 5, paragraph 10 of the Office Action rejects claims 1, 2, 6, 7, 12-17, 67 and 68 under 35 U.S.C. § 102(b) as being anticipated by Huang et al.

Huang et al. shows data obtained from a screen of a library of CpG island (CGI) loci. They describe their procedure as differentiated methylation hybridization (DMH), and this procedure is illustrated in Figure 2 on p. 461. This figure shows *Mse* I digestion, ligation to linkers, and *Bst*UI digestion, to prepare the CGI library. Further, Huang very clearly states that, "…genomic fragments (200-300Bp) derived from CpG island clones…" are of a particular length (Fig. 6 legend, p. 465) in a Southern blot.

In contrast to Huang et al., the invention of claim 1 as here amended, and of claims dependent on claim 1, do not describe a Southern blot, nor a DMH using a CGI library. The present invention generates a molecular profile of a genome using immobilized genomic probes at known locations that represent for example an entire or substantially an entire genome or an entire chromosome.

The legal standard for rejection of a claim under 35 U.S.C. § 102 is one of strict identity. Huang et al. is clearly different from the invention of claim 1, therefore claim 1 and claims 2, 6, 7, 12-17 and 67-68 dependent on claim 1, which include all of the limitations of claim 1, are novel in view of Huang et al. Applicants respectfully request that the Examiner withdraw rejection of claims under 35 U.S.C. § 102(b) on the basis of Huang et al.

Cronin et al. (Human Mutation 7: 244-255, 1996)

Claims 1-8, 12, 14-17, 67 and 68 are rejected under 102(b) as anticipated by Cronin et al. The Examiner states that Cronin et al. teach detection of cystic fibrosis mutations by hybridization of target nucleic acids to an array of immobilized probes.

However, Cronin et al. show DNA sequence analysis (Abstract). Cronin shows two types of arrays, a first arry with 428 probes and a second with 1480 probes. The arrays are synthesized by photolithography, the probes having short sequences that differ by one nucleotide, and are used to determine in samples the sequence of only a single exon, exon 11, of only one gene, cystic fibrosis transmembrance conductance regulator (CFTR), to detect mutated sequences. For this purpose, all of the photolithographed immobilized sequences are based on the sequence of only this one exon (see Cronin et al., Figure 1, p. 245).

As the CFTR gene has at least 10 more exons, a human genome that contains about 30,000 genes, each gene having only about 10 exons (an underestimate), if analyzed by the method of Cronin et al. would require that the user provide photolithographed probes with immobilized nucleic acids in enormous numbers. The number of probes would be more than 128,400,000 (428 x 30,000 x 10) using the first method, and more than 444,000,000 elements (1,480 x 30,000 x 10) using the second method, for analysis of a sequence of all or substantially all of a human genome.

Cronin's method is clearly not the same as the method of claim 1. In contrast to Cronin, claim 1 as here amended provides a method for generating a molecular profile, not a sequence, of a genomic target. No sequence information of the nucleic acids of the samples is required, nor is any particular sequence being determined by the user of the method of claim 1, so that no input sequence is photolithographed onto the probe. In contrast to Cronin, the methods of the claims

herein require a plurality of nucleic acid probes comprising a plurality of immobiled nucleic acid segments. The term "a molecular profile of genomic DNA" means detecting fragments in a test sample of nucleic acid representing a genomic DNA, compared to a control (e.g., "normal") sample of DNA (see p. 14, lines 23-25 of the application as filed), that hybridize to the same set of probes. In contrast to what is described in Cronin, an entire genome or one or more entire chromosomes can be analyzed by the methods herein, because the immobilized probes of claim 1 contain nucleic acid from a collection of clones (BACs, PACs, YACs) collectively having nucleic acid covering such a span (see for example p 16, lines 13-19).

Because Cronin demands obtaining a sequence from the sample, the method of Cronin et al. is inoperative for an entire genome, unless practiced by a very large number of users over a very long period of time. Because of these differences, Cronin's method teaches away from present claim 1 and claims that depend from claim 1 directly or indirectly.

The legal standard for rejection under 35 U.S.C. § 102 is one of strict identity. Cronin et al. is a sequencing method used for a short portion of one gene, and is not the same as the present invention of claim 1 and its dependent claims, which is a molecular profile of genomic DNA for an entire genome or substantially an entire genome. Therefore this reference is not proper prior art to reject claim 1 and claims that depend from claim 1 under 35 U.S.C. § 102(b).

Applicants respectfully request that the Examiner withdraw rejection of claims in view of Cronin et al.

Issues under 35 U.S.C. §103

Cronin et al. (Human Mutation 7: 244-255, 1996) and Waggoner et al. (U.S. patent 5,268,486)

Claim 9 is rejected by the Examiner on p. 7, paragraph 13 of the Office Action, under 35 U.S.C. §103 for allegedly being obvious in view of the combination of Cronin et al. in combination with Waggoner et al.

The legal standard for a proper rejection under 35 U.S.C. §103(a), is that the references, either alone or in proper combination, must teach or suggest all of the claim limitations of Applicant's claimed invention. Applicant will show that the deficiencies of Cronin are not cured

by Anderson. Accordingly, Applicants will show that a prima facie case of obviousness has not been established and that the rejection can be properly withdrawn.

Claim 9 is directed to sample target nucleic acid having detectable label that comprises Cy3™ or Cy5™. However, Cronin does not teach Cy3™ and Cy5™, and in fact uses only fluorescein (see Cronin, p. 246, right column). The Office Action alleges that Waggoner et al. teaches Cy3™ and Cy5™ . In fact, Waggoner shows a very large number of dyes (e.g., columns 13-16 and claim 1) having a large variety of substituents at $R_1 - R_7$. A user of the present invention, upon reading Waggoner, would not know how to choose one preferred dye, let alone both Cy3™ and Cy5™, from the large number of dyes disclosed in this reference. Neither Cronin nor Waggoner remedies the defect of the other, therefore these references in combination do not teach or suggest claim 9, and claim 9 is not obvious in view of this combination of references.

Further, neither Cronin nor Waggoner teaches nor suggests all of the limitations of claim 1, such as providing a plurality of nucleic acid probes comprising a plurality of immobilized nucleic acid segments, which are fragments of genomic nucleic acid, and contacting the probes with fragments of genomic nucleic acid having a length smaller than about 200 bases. Since neither supplies all of these requirements of claim 1, neither of them supplies all of the limitations of claim 9, which depends indirectly from claim 1. Cronin et al. as shown above does not show the invention of claim 1, therefore does not remedy these defects of Waggoner et al. Finally, Cronin et al. published in 1996 does not provide any motivation to make a combination with Waggoner et al., published in 1993, such as a reference to Waggoner et al.

For any of these reasons, it would not have been obvious to one of ordinary skill in the art at the time the invention was made to combine Cronin et al. with Waggoner et al. to obtain the method of claim 9. Applicants respectfully request that the Examiner withdraw rejection of claim 9 in view of the combination of Waggoner et al. and Cronin et al.

Cronin et al. (Human Mutation 7: 244-255, 1996) and Anderson et al.(Nucl. Acids. Res. 9: 3015-3027, 1981)

The Office Action on p. 7, paragraph 14 alleges that claim 10 is obvious in view of Cronin et al. further in view of Anderson et al. Claim 10 as amended herein requires

fragmenting the sample of target genomic nucleic acid to sizes smaller than about 200 bases by digestion with DNase enzyme.

Anderson et al. is a method for DNA sequencing of libraries of cloned randomly-fragmented (shotgun) DNA, which has been fragmented by limited attack by DNase I.

The phrase "enzymatic digestion, e.g., DNase enzyme or equivalent" (see the specification as filed, p. 2, lines 24-26) includes any enzyme capable of fragmenting a nucleic acid, of which an DNase which has an endonuclease activity is exemplary, and this phrase includes restriction enzymes. See also p. 23, lines 20-22, in which an example of a DNase which is a restriction enzyme, *Cvi* JI, is given. The claim is not limited to DNase I.

The Examiner on p. 8 of the Office Action alleges that "Anderson teaches fragmentation of genomic DNA to sizes below 200 base pairs by digestion with 2.2 ng or more of DNase I (Figure 1)." However lane F of Anderson's Figure 1, shows no particular band or smear visible in the size range of less than 200 base pairs (i.e., below the 312 base pair marker shown in lane I).

Further, Anderson specifically states, "...fractions containing DNA fragments in the range 300-1000 bp were pooled." [p. 3019, last line, to p. 3020, first line; emphasis added]. In specifically pointing out a procedure to obtain fragments in the size range 300-1000 base pairs, Anderson teaches away from the limitation of claim 10, which requires that the size range be less than 200 bases.

Cronin et al. do not teach fragmentation of DNA by DNase digestion, as pointed out on page 8 of the Office Action, therefore Cronin does not remedy this deficiency in Anderson. Further, Cronin published in 1996 does not reference Anderson published in 1981, therefore provides no motivation for combining any of its teachings with Anderson. Even had Cronin referenced Anderson, Anderson fails to provide the size range required of claim 10 and teaches away from that size range.

For these reasons, one of ordinary skill in recombinant DNA technologies would not be motivated to combine Cronin with Anderson to produce the invention of claim 10. Applicants respectfully request that rejection of claim10 under 35 U.S.C. § 103 be withdrawn.

Cronin et al. (Human Mutation 7: 244-255, 1996), Anderson et al.(Nucl. Acids. Res. 9: 3015-3027, 1981) and Ordahl (Nucl. Acids Res. 3: 2985-99, 1976).

The Examiner on p. 8, paragraph 15 rejects claim 11 in view of Cronin et al. in combination with Ordahl and Anderson. Claim 11 is directed to an embodiment of the present invention that uses mechanical forces of shearing followed by DNase enzyme digestion to obtain fragments of less than 200 bases.

Applicants show above that Cronin et al. is inapt as a reference, because Cronin is a method to obtain nucleotide sequences of merely one exon of one gene, and because this method requires ab initio providing short overlapping immobilized sequences in order to probe a target sample to obtain the exact sequence of the related portion in that sample. Cronin et al. unlike the invention of the present claims does not provide a molecular profile of a genomic nucleic acid sample as defined in the presen specification.

Anderson et al., like Cronin as discussed above, is also a method of sequencing, in Anderson's case a method of sequencing DNA obtained in shotgun clones. Anderson like Cronin does not provide any of the limitations of claim 1 from which claim 11 depends, and therefore does not teach the method of claim 11.

Ordahl et al. fails to remedy the deficiencies of Anderson and Cronin. Ordahl et al. is merely a method of shearing DNA, and like Anderson and Cronin, fails to provide the limitations of claim 1.

Further, neither Anderson et al. (1981) nor Ordahl et al. (1976) is cited by Cronin et al. (dated 1996) in the methods sections on pp. 246-248. Since Cronin et al. uses photolithographically-synthesized polynucleotides having predetermined sequences to make an array, Cronin et al. is a different art than Anderson et al. and Ordahl et al.

One of ordinary skill in the art of genomic profiling would have no motivation to combine two different sequencing methods with a paper on mechanical shearing, and even if one had such motivation, the combination fails to produce the limitations of present claims 1 and present 11. One would have no motivation to combine either Cronin or Anderson with Ordahl,

and even if one had motivation, no one of many combinations of these papers is the method of present claim 11.

Applicants respectfully request that the Examiner withdraw rejection of claim 11 under 35 U.S.C. § 103.

CONCLUSION

Applicants respectfully request that the Examiner reconsider the application and claims in light of the foregoing reasons and amendments, and submit that the present claims are in condition for allowance.

If in the Examiner's opinion, a discussion would be helpful in prosecution of the present application, Applicants invite and encourage the Examiner to contact their representative at the telephone number below.

Applicants believe that, except for the extension of time fee, no additional fees are necessitated by the present Response. However, in the event any such fees are due, the Commissioner is hereby authorized to charge Deposit Account No. 50-0311 (27476-504).

Respectfully submitted,

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